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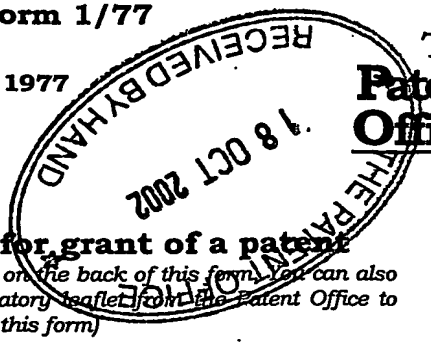

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3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	**NOVARTIS FORSCHUNGSSTIFTUNG ZWEIGNIEDERLASSUNG FRIEDRICH MIESCHER INSTITUTE FOR BIOMEDICAL RESEARCH MAULBEERSTRASSE 66, CH-4058 BASEL SWITZERLAND**
	Patent ADP number *(if you know it)*	82065830 01
	If the applicant is a corporate body, give the country/state of its incorporation	**SWITZERLAND**
4.	Title of invention	**MODULATION OF S6 KINASE ACTIVITY FOR THE TREATMENT OF OBESITY**
5.	Name of your agent (if you have one) "Novartis Pharmaceuticals UK Ltd Patents and Trademarks Wimblehurst Road Horsham West Sussex RH12 5AB" Patents ADP number (if you know it)	**B.A. YORKE & CO. CHARTERED PATENT AGENTS COOMB HOUSE, 7 ST. JOHN'S ROAD ISLEWORTH MIDDLESEX TW7 6NH 1800001**

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Signature: B. A. Yorke & Co.

Date: 18 October 2002

B.A. Yorke & Co.

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MODULATION OF S6 KINASE ACTIVITY FOR THE TREATMENT OF OBESITY

The current invention relates to obesity, in particular the treatment of obesity with modulators of S6 kinase (S6K) activity.

BACKGROUND OF THE INVENTION

Obesity is a metabolic disease frequently associated with insulin resistance and together constitute risk factors for later development of type II diabetes and cardiovascular diseases. Insulin resistance occurs well before development of type II diabetes, and insulin is overproduced to compensate for the insulin resistance and to maintain normal glucose levels. Insulin resistance can later develop into type II diabetes, as the pancreas can no longer produce enough insulin to maintain normal glucose levels. Early stages of type II diabetes are associated with elevated levels of insulin but as the disease progresses the pancreas may fail to produce insulin, resulting in increased blood glucose levels. Diabetes is a significant risk factor for both heart disease and stroke and is the leading cause of blindness and end-stage renal failure.

There are over 1 billion overweight individuals worldwide with 100 million clinically obese. The increasing health care costs of treating obesity related diseases in the US alone are estimated at over $100 billion annually. Current methods for treating obesity include behavioural modification, dieting, surgery (gastroplasty), administering pharmaceutical agents that block appetite stimulating signals or absorption of nutrients (fat), and administering agents that increase thermogenesis or fat metabolism. Some of these methods have disadvantages in that they rely on patient resolve, are invasive, or have unwanted side effects. There remains a need for non-invasive therapies to promote weight loss in obese individuals. An understanding of the mechanisms by which obesity is regulated may provide important therapeutic information.

Intracellular signaling pathways have been implicated in various cellular functions and the involvement of kinases in such pathways provides potential drugable targets for modulating a signalling pathway. Much work has been carried out in attempting to delineate the S6 kinase (S6K) signalling pathway. S6 kinase is a kinase that phosphorylates the ribosomal protein, S6. S6K1-deficient mice are viable and fertile but exhibit a conspicuous reduction in body size during embryogenesis, an effect mostly overcome by adulthood. The small size of the homozygous mutant mice is consistent with a defect in translational capacity (Shima et al., 1998, EMBO J., 17, 6649-6659). Mice deficient for S6K1 are hypoinsulinaemic and mildly glucose intolerant, not due to a lesion in glucose sensing or insulin production, but to a reduction in pancreatic endocrine mass, which is accounted by a selective decrease in beta-cell size. Thus, hypoinsulinaemia and not insulin resistance is thought to be the primary lesion predisposing S6K1-deficient mice to hyperglycaemia. The observed phenotype closely parallels the form of preclinical type 2 diabetes mellitus, in which malnutrition-induced hypoinsulinaemia predisposes individuals to glucose intolerance (Pende et al., 2000, Nature, 408, 994-997).

Deletion of the S6K1 gene in mice led to the identification of an S6K1 homologue, S6K2, which can partly compensate for S6K1 function biochemically to phosphorylate S6 (Shima et al., 1998, EMBO J., 17, 6649-6659). The precise function of S6K2 in vivo is not known to date. S6K activity has, however, been implicated in cancer and angiogenesis.

SUMMARY OF THE INVENTION

In accordance with a first aspect of the invention, a method of identifying an agent effective in treating weight disorders is provided, the method comprising

the steps of: i) incubating S6 kinase with a compound; ii) detecting S6 kinase activity; and iii) determining a compound-induced modulation in the S6 kinase activity relative to when the compound is absent, wherein an alteration of the S6 kinase activity in the presence of the compound is indicative of an agent effective in treating weight disorders. The compound -induced modulation is preferably independent of an effect of mammalian Target of Rapamycin (mTOR) activity. In one embodiment, the modulation is inhibition of S6 kinase 1 activity and the weight disorder is obesity or an overweight condition. In an alternative embodiment, the modulation is activation of S6 kinase 1 activity and the weight disorder is an underweight condition. S6 kinase activity can be conveniently assayed using S6 as a substrate and is easily amenable to high throughput assays.

Also provided by the invention are methods of screening for an agent effective in treating weight disorders, comprising contacting transcriptionally active cellular components with a nucleic acid encoding an S6K gene operably linked to a promoter sequence or an S6K promoter sequence operably linked to a reporter gene in the presence of at least one compound; and detecting an effect of the compound on S6 kinase expression or S6 kinase promoter activity, wherein detection of a decrease or an increase in S6 kinase expression or promoter activity is indicative of an agent effective in treating weight disorders. Such assays can be cell-based assays, where the transcriptionally active cellular components and nucleic acid is present in a cell. In preferred embodiments, the S6 kinase is S6 kinase 1.

The invention further provides methods of identifying an agent effective in treating weight disorders, comprising: providing a non-human animal comprising an S6 kinase gene; administering a compound to the non-human animal; and determining whether weight loss or gain is affected relative to when the compound is absent. The S6 kinase gene can be from the same or different

species as the transgenic animal (for example, a mouse comprising an S6 kinase gene derived from human sequences).

Also encompassed are agents identified by the methods of the invention.

In a further aspect, a method for reducing adipocyte size is provided, comprising contacting an adipocyte with an effective amount of an S6 kinase 1 inhibitor.

In yet another aspect, methods for treating a weight disorder, comprising administering to a subject a pharmaceutically effective amount of an S6 kinase modulator are provided. The S6 modulator can be an S6K1 inhibitor, in particular where the weight disorder is obesity or an overweight condition.

Thus, also provided are specific modulators of S6 kinase for the manufacture of a medicament for the treatment or prophylactic treatment of a weight disorder, such as a specific inhibitor of S6 kinase 1 for the treatment or prophylactic treatment of an overweight condition, such as obesity.

Similarly, also provided are modulators of S6 kinase activity for use in treating weight disorders, such as an inhibitor of S6 kinase 1 activity for use in the treatment of an overweight condition, such as obesity.

In a further aspect of the invention, a method of diagnosing a predisposition to a weight disorder is provided, comprising: obtaining a sample from an individual, detecting the level of S6 kinase activity, preferably S6 kinase 1 activity, in the sample and correlating a change in S6 kinase activity in the sample when compared to a normal control value or range of values with a predisposition to a weight disorder. For example, an increase in S6 kinase 1 activity when compared to a normal control value or range of values is indicative of a predisposition to obesity.

Also provided are methods of evaluating treatments for weight disorders, the method comprising administering a therapeutic agent to a non-human animal comprising an S6 kinase gene, in particular S6K1, and determining the effect of the agent on weight gain or loss.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based upon the finding that S6K1 deficient mice maintain their lower body weight relative to wild type mice as they age, even when placed on a high fat diet. S6K1 deficient mice eat the same total amount of food, but compared to body weight, they eat much more. Dissection of S6K1 deficient mice reveals a severe reduction of white fat and brown fat (e.g., in epidedymal fat), whereas organ size is unaffected. The reduction in fat is due to a reduction in fat cell (adipocyte) size. Thus, it is expected that modulation of S6K1 activity will be useful in treating weight disorders. Although mTOR (mammalian target of rapamycin, which phosphorylates and activates S6 kinase) can be targeted directly to modulate S6K1 activity, direct targeting of S6K1 avoids more general side-effects of inhibiting mTOR activity, thereby providing more specificity for the treatment of patients with weight disorders.

Accordingly, the present invention provides a method of identifying an agent effective in treating weight disorders, such as obesity, based on the modulation of S6 kinase activity, in particular S6 kinase 1 activity. Typically such a method will comprise the steps of incubating S6 kinase (or a functional equivalent or derivative thereof) with a compound; detecting S6 kinase activity; and determining the compound-induced modulation in the S6 kinase activity relative to when the compound is absent. An alteration of the S6 kinase activity in the presence of the compound is indicative of an agent effective in treating weight disorders.

Unless otherwise clear from the context, "S6K" or "S6 kinase" is used herein to encompass both S6K1 and S6K2 (see, for example, Genebank Accession No. M57428, AJ007938, AB019245, NM003952 and related sequences), although S6K1 is preferred. Exemplary functional equivalents or derivatives of S6K include molecules where S6K is covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid. Derivatives that retain common structural features can be fragments of S6K, in particular fragments maintaining catalytic activity or isoform specific characteristics. For example, the carboxy- terminal sequences of S6K1 and S6K2 exhibit only about 20% identity and it therefore may be useful to include such isoform-specific features in fragments when isoform-specific assays are desired. Preferably, fragments will be between 50 and 350 amino acids in length. Derivatives of S6K also comprise mutants thereof, which may contain amino acid deletions, additions or substitutions, subject to the requirement to maintain at least one feature characteristic of S6K, preferably catalytic activity. Thus, conservative amino acid substitutions may be made substantially without altering the nature of S6K, as may truncations. Additions and substitutions may moreover be made to the fragments of S6K used in the screening methods of the invention, in particular those enhancing S6K catalytic activity or providing some other desirable property. For example, T389, T229 and S371 in mouse S6K1 (also known as p70S6K) are homologous to T389, T238 and S380 in Drosophila p70S6K. T389 is particularly indicated for mutation to an acidic amino acid residue in order to produce a constitutively active kinase.

The screening assays of the invention are not limited to any particular method of determining S6 kinase activity. S6 kinase assays are well known in the art (see for example USPN 6,372,467, which is herein incorporated by reference in its entirety). Briefly, S6 kinase will be incubated with a suitable substrate, such as S6, in a buffer allowing phosphorylation of S6. Phosphorylation of the substrate can be detected using a labelled phosphate group, such as the use of the radioactive label ^{32}P present as the ATP source in the buffer. Alternatively,

antibodies specific for the phosphorylated products of S6K catalytic activity can be used to detect activity. As will be apparent to those of ordinary skill in the art, the assays are easily amenable to high through-put technologies using robotics and automated processes.

S6K activity can also be assayed by detecting downstream targets of the kinase. For example, S6K is known to affect transcription and translation of specific targets, such as genes with polypyrimidine tracts (5'TOPs) and ribosomal genes. (Fumagalli S, Thomas G. (1999) Ribosmal Protein S6 Phosphorylation and Signal Transduction. In: Translational Control. Eds. Hershey, J, Mathews, M, Sonenberg, N. Cold Spring Harbor Press. pp 695-717). Assays specific for S6K1 can also be designed by detecting binding of specific proteins, such as nerabin to the C-terminal domain of S6K1, as is well known in the art (Burnett PE, Blackshaw S, Lai MM, Qureshi IA, Burnett AF, Sabatini DM, Snyder SH. Neurabin is a synaptic protein linking p70 S6 kinase and the neuronal cytoskeleton. Proc Natl Acad Sci U S A. 1998 Jul 7;95(14):8351-6).

A compound-induced modulation of S6K activity means that there is a change in S6K activity (or expression) in the presence of the compound relative to when the compound is absent. In particular a compound induced inhibition of S6K activity is reflected by a decrease in S6K activity relative to when the compound is absent. Conversely, a compound induced activation of S6 activity is reflected by an increase in S6K activity.

Activators and inhibitors are referred to collectively herein as modulators and preferably influence the kinase activity of S6K directly. Assays carried out using reconstituted components can be easily designed to achieve direct S6K inhibition (i.e., specific inhibition of S6K catalytic activity and not inhibition of the formation of active kinase, for example, through the action of mTOR). Typically, S6 kinase 1 activity will be specifically inhibited, in particular when inhibition of S6 kinase 1 signaling in adipose tissue and weight loss is desired.

In accordance with a further aspect of the invention, a method is provided for screening an agent effective in treating weight disorders, by identifying compounds that modulate expression of an S6K gene or a gene expressed under the control of S6K regulatory sequences.

The methods comprise contacting transcriptionally active cellular components, preferably in a cell, with a nucleic acid encoding an S6K gene operably linked to a promoter sequence or an S6K promoter sequence (or other S6K regulatory regions allowing expression of the reporter gene) operably linked to a reporter gene in the presence of at least one compound; and detecting an effect of the compound on expression of the coding region, be it S6 kinase expression or reporter gene expression. A decrease or an increase in S6 kinase expression or promoter activity is indicative of an agent effective in treating weight disorders. Such assays can be cell-based assays, where the transcriptionally active cellular components and nucleic acid is present in a cell, although in vitro transcription assays are also well known in the art. In preferred embodiments, the S6 kinase is S6 kinase 1.

The reporter gene encodes any molecule capable of providing a detectable change. Such reporter molecules include fluorescent moieties (e.g., fluorescent proteins, such as, cyan fluorescent protein, CFP; yellow fluorescent protein, YFP; blue fluorescent protein, BFP; or green fluorescent protein, GFP; all available commercially, Clontech Living Colors User Manual, antigens, reporter enzymes and the like. Reporter enzymes include, but are not limited to, the following: beta-galactosidase, glucosidases, chloramphenicol acetyltransferase (CAT), glucoronidases, luciferase, peroxidases, phosphatases, oxidoreductases, dehydrogenases, transferases, isomerases, kinases, reductases, deaminases, catalases and urease. In selecting a reporter molecule to be used in the presently claimed method, the reporter molecule itself should not be inactivated by any putative agent or other component present in the screening assay,

including inactivation by any protease activity present in the assay mixture. The selection of an appropriate reporter molecule will be readily apparent to those skilled in the art.

The nucleic acid will typically be provided in a vector allowing replication in one or more selected host cells, as is well known for a variety of bacteria, yeast, and mammalian cells. For example, various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, phage, or any other suitable vector or construct which can be taken up by a cell and used to express the sequence of interest or reporter gene.

Expression vectors usually contain a promoter operably linked to the protein-encoding nucleic acid sequence of interest, so as to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known, as are the S6K1 and S6K2 promoters (upstream regulatory sequences). "Operably linked" means joined as part of the same nucleic acid molecule, suitably positioned and oriented for transcription to be initiated from the promoter. DNA operably linked to a promoter is "under transcriptional control" of the promoter. Transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems. Expression vectors of the invention may also contain one or more selection genes, such as genes conferring resistance to antibiotics or other toxins.

The methods of the invention may therefore further include introducing the nucleic acid into a host cell. The introduction, which may (particularly for in vitro introduction) be generally referred to without limitation as "transformation", may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccini, as is well known in the art. See, for example, Keown et al., Methods in Enzymology, 185:527 537 (1990) and Mansour et al., Nature 336:348-352 (1988).

Host cells transfected or transformed with expression or cloning vectors described herein may be cultured in conventional nutrient media. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in "Mammalian Cell Biotechnology: a Practical Approach", M. Butler, ed. JRL Press, (1991) and Sambrook et al, supra.

The invention further provides methods of identifying an agent effective in treating weight disorders, administering a compound to a non-human animal having an S6 kinase gene and determining whether weight loss or gain is affected relative to when the compound is absent. The non-human animals will typically be laboratory mammals such as mice or rats and various doses can be administered orally mixed with feed or by any other appropriate means, which may be chosen dependent on the properties of the compound, such as stability and targeted delivery. The S6 kinase gene may be from a different species as the laboratory mammal, for example, the use of a mouse comprising a human S6K gene, which replaces the mouse S6K gene, will be particularly useful to determine the effects of agents on human S6K without using human subjects.

The screening system is preferably used to screen compounds that may be present in small molecule libraries, peptide libraries, phage display libraries or natural product libraries. Compound may be inorganic or organic, for example, an antibiotic or antibody. For ease of administration, the compound is preferably a small molecule, which might bind to the catalytic site or ATP binding site. For isoform-specific inhibition, the compound may interfere with the C-terminal domain of S6K, which is not highly conserved between S6K1 and S6K2.

In order to potentially improve S6K modulators, isolated S6K can be used to establish secondary and tertiary structure of the whole protein or at least of the areas responsible for the enzymatic activity. Conventional methods for the identification of the 3-dimensional structure are, for example, X-ray studies or NMR studies. The data obtained with these or comparable methods may be used directly or indirectly for the identification or improvement of modulators of S6K, such as to provide specificity between S6K1 and S6K2. A commonly used method in this respect is, for example, computer aided drug design or molecular modelling.

Kits useful for screening such compounds may also be prepared in accordance with the invention, and will comprise essentially S6K or a fragment thereof useful for screening, and instructions. Typically the S6K polypeptide will be provided together with means for detecting S6K activity and at least one compound (putative agent).

S6K for use in kits according to the invention may be provided in the form of a protein, for example in solution, suspension or lyophilised, or in the form of a nucleic acid sequence permitting the production of S6K or a fragment thereof in an expression system, optionally in situ.

Compounds according to the invention may be identified by screening using the techniques described hereinbefore, and prepared by extraction from natural or

genetically modified sources according to established procedures, or by synthesis, especially in the case of low molecular weight chemical compounds. Proteinaceous compounds may be prepared by expression in recombinant expression systems, for example a baculovirus system, or in a bacterial system. Proteinaceous compounds are mainly useful for research into the function of signalling pathways, although they may have a therapeutic application, such as humanized inhibitory antibodies directed against S6 kinase 1. Alternatively, nucleic acids, such as siRNA can be administered to inhibit S6K1 activity. SiRNA technology can be routinely applied based on sequences specific for S6K1, such as AGTGTTTGACATAGACCTG or preferably AAGGGGGCTATGGAAAGGTTT. Targeted expression of siRNAs can be achieved using tissue-specific promoters, such as promoters specific for adipose tissue.

Low molecular weight compounds, on the other hand, are preferably produced by chemical synthesis according to established procedures. They are primarily indicated as therapeutic agents. PKC inhibitors, or derivatives or modifications thereof, may be used as potential agents effective in specifically inhibiting S6K and in treating obesity or other weight disorders. Low molecular weight compounds and organic compounds in general may be useful as agents for use in the treatment of obesity (or conditions associated with weight disorders).

The present invention also provides a method for reducing adipocyte size comprising contacting an adipocyte with an effective amount of an S6 kinase 1 inhibitor. Thus, also provided by the invention are compounds that directly modulate S6 kinase activity for use in treating weight disorders, such as obesity. In particular, compounds that directly inhibit S6 kinase 1 activity for use in treating overweight conditions, such as obesity are provided. Obesity is normally defined through the determination of the Body Mass Index (BMI), which is calculated using a simple formula (weight in kg divided by the square of height in meters). A calculated BMI of 18.5 to 24.9 is considered to be normal weight; a BMI of 25-29.9 kg/m2 indicates overweight; a BMI of 30-39.9 indicates obesity;

and a BMI of 40 and upwards indicates morbid obesity. Similarly, a calculated BMI of less than 18.5 may be considered an underweight condition.

S6K modulators (e.g., inhibitors) for use in treating weight disorders (e.g. overweight conditions, such as obesity) may be formulated according to conventional methodology, depending on the exact nature of the modulator, and will typically comprise the modulator or a precursor thereof in association with a biologically acceptable carrier. In considering various therapies, it is understood that such therapies may be targeted to tissues demonstrated to express S6K1, in particular to adipocytes.

Delivery of the modulator to the affected cells and tissues can be accomplished using appropriate packaging or administration systems. For example, the modulator may be formulated for therapeutic use with agents acceptable for pharmaceutical administration and delivered to the subject by acceptable routes to produce a desired physiological effect. An effective amount is that amount that produces the desired physiological effect, such as, weight loss.

In a further aspect of the invention, the invention also provides a specific inhibitor of S6 kinase for the manufacture of a medicament for the treatment or prophylactic treatment of obesity.

The invention also provides a method of diagnosing a predisposition to a weight disorder, comprising: obtaining a sample from an individual, detecting the level of S6 kinase, preferably S6 kinase 1, in the sample and correlating a change in the amount of S6 kinase in the sample when compared to a normal control value or range of values with a predisposition to a weight disorder. The presence of S6 kinase can be easily determined using antibodies or using activity assays as described above. Antibodies specific for S6K2 can be used as a control when S6K1 specific measurements are desired. For example, an increase in S6 kinase 1 activity of at least 20%, preferably at least 30% when compared to a normal

control value or range of values is indicative of a predisposition to obesity. The sample may be any tissue sample or body fluid, but is preferably adipose tissue.

Also provided are methods of evaluating treatments for weight disorders, the method comprising administering a therapeutic agent to a non-human animal comprising an S6 kinase gene, in particular S6K1, and determining the effect of the agent on weight gain or loss. Alternatively, a sample, such as adipose tissue or peripheral blood can be withdrawn and tested for S6K levels or activity levels to establish a modulatory effect on S6K.

In addition, methods of evaluating treatments for weight disorders are provided, comprising administering a therapeutic agent to a non-human animal deficient in S6 kinase (such as a knock-out animal), in particular deficient in S6K1, and determining the effect of the agent. Such methods can be used to establish whether any unwanted side effects of the therapeutic agent are present.

The invention is further described, for the purposes of illustration only, in the following examples.

EXAMPLES

Methods of molecular genetics, protein and peptide biochemistry and immunology referred to but not explicitly described in this disclosure and examples are reported in the scientific literature and are well known to those skilled in the art. For example, standard methods in genetic engineering are carried out essentially as described in Sambrook et al., Molecular Cloning: A laboratory manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY, 1989.

Example 1:S6K1 deficient mice are smaller than wild type

S6K1 deficient mice were previously shown to have a reduction in body size during embryogenesis but the effect was thought to be mostly overcome by adulthood. This Example demonstrates that as they age, S6K1 deficient mice maintain a lower body weight relative to wild type mice.

S6K1 deficient mice were generated as described by Shima et al. (1998, EMBO J., 17, 6649-6659). Mice were maintained and housed in groups of 12 (in cages of 3) and maintained on a 12 hour light/12 hour dark cycle (lights on at 06:00 GMT). Body weight was recorded weekly in wild type (wt) and S6K1 deficient mice fed regular chow diet. The data are provided in Tables I and II below and show that S6K1 deficient mice are smaller and have lower body weight compared to wt.

Table I (body weight wild type mice)

Age (Weeks)	10	11	12	13	14	15	16	17	18	19	20
Wt mouse 1	26.4	28.4	28.3	28	28.8	30.2	30.2	29.7	29.8	30.8	31
2	24.7	26.5	27.4	29	28.8	30.2	29.5	25.5	died		
3	25.2	26.6	25.6	26	26.4	27.7	27.8	28.8	29.4	30.5	30
4	26.5	28.3	28.2	28.6	29	30.8	31.2	25.3	died		
5											
6	24.5	28.3	28.5	31.8	32.1	34.2	35.2	36.3	36.7	37.6	37.7
7	25.2	26.6	28	30.1	29.3	30.1	30.4	30.7	31.6	32	31.6
8	20.2	20.3	20.5	20.9	20.5	21.3	21.9	22.4	23	23.6	22.8
9	25.9	27	27.4	28.9	28	29.6	30	31.1	31.7	32.5	31.9
10	25.9	26	29.5	32.1	31.1	32.6	32.3	34.7	33.6	35.3	36.5
11	28.8	29.8	32	35.1	36.8	38.1	40.4	41.7	42.4	44.1	44.5
12	28	32	33.5	35.3	36.4	36.6	37.6	38.1	40	40.1	40.5
13	26.3	29.3	31	33.8	33.5	34.9	35.1	36.1	36.5	36.9	38.5
14	26.5	28	29	29.6	29.2	30.7	31.3	31.5	32.8	33.7	34
15	26.5	29	30.1	30.4	30.8	32.4	32.3	32.4	33.5	33.6	33.3
Average	**25.8**	**27.6**	**28.5**	**30.0**	**30.0**	**31.4**	**31.8**	**31.7**	**33.4**	**34.2**	**34.4**
S.D	2.0	2.6	3.1	3.8	4.1	4.1	4.5	5.3	5.1	5.2	5.7

Table I (continued)

Age (Weeks)	21	22	23	24	25	26	27	28	29	30
Wt mouse 1	32	32.4	32.2	32.4	33	33.1	33.2	33.3	33.5	33.6
2		Died								
3	30	30.8	30.9	31	32	32.8	32.5	32.6	33.4	33.9
4		Died								
5								died		
6	38	39.5	39.8	38.7	38.8	38.9	39	39.2	39.7	40.9
7	33	34.1	34.2	33.5	33.1	33.1	33.1	33.2	33.4	33.9
8	24	24.2	23.5	23.4	23.5	23.8	died			
9	33	34	34	34.1	34.1	34.1	34	33.5	33.2	33.2
10	35.9	36.1	36.2	35	35	35	34.2	33.4	34.4	34.4
11	45	46.2	45.2	44.8	44.5	44.6	44.3	42.4	42	42.1
12	40.6	40.8	40.4	39	39.1	39.2	38	37.6	37.4	37.9
13	40	40.2	39.8	39.4	39.4	39.6	39.4	39.5	39.5	39.7
14	34.5	34.7	34.5	34	34	33.9	33.4	33	32.7	32.8
15	35.9	36.2	35.8	35.5	35.5	35.6	35.1	34.9	34.3	34.5
Average	**35.2**	**35.8**	**35.5**	**35.1**	**35.2**	**35.3**	**36.0**	**35.7**	**35.8**	**36.1**
S.D	5.5	5.6	5.5	5.3	5.2	5.1	3.7	3.4	3.3	3.4

Table II (body weight S6K1 -/-)

Age (weeks)	10	11	12	13	14	15	16	17	18	19	20
S6K1 -/- 1	22	24.3	24.6	26.1	26.1	27	27.3	29.2	28.8	28.9	28.8
2	23.1	24	26	27.1	27.5	28.3	27.7	29.3	29	30	30.1
3	22	25	26.3	26.4	26.3	27.7	28.3	29.8	30.5	31.2	31
4	21	22.8	24.4	25.1	27	27.4	24.7	25.9	26.5	26.9	27.9
5	22.2	23.6	24.4	25.5	25	25.8	24.6	26.1	26.1	26.5	27.2
6	23	24.5	25.6	26.9	25.2	25.2	27.1	27.8	28.1	28.5	28.3
7				died							
8	23.5	24.6	25.6	26.5	26.3	26.5	26.4	27.1	27	27.3	27.2
9	20.2	22.1	24.3	25	25.9	26.3	26.4	26.7	27.2	27.2	27.2
10	19.9	22	23	24.9	25.1	25.4	25.3	26	26.5	27.7	27.4
11	19.6	20.3	22	23.3	22.7	24.3	24.1	25	24.9	26.3	25.9
12	22	23.4	24.5	25.8	25.6	26.6	26.7	27.8	27.3	27.9	27.7
13	21.2	22.3	22.6	23.6	24.7	25.5	25.5	26.4	26.5	27.2	27.8
14	21.5	23	23.4	23.8	25	26.4	26	27.5	28	28	died
15	19.2	19.8	20.9	21.3	20.1	22	20.6	21.8	21.4	21.3	25.5
average	**21.5**	**23.0**	**24.1**	**25.1**	**25.2**	**26.0**	**25.8**	**26.9**	**27.0**	**27.5**	**27.8**
S.D	1.3	1.6	1.6	1.6	1.9	1.6	1.9	2.0	2.1	2.2	1.5

Table II (continued)

Age (weeks)	21	22	23	24	25	26	27	28	29	30
S6K1 -/- 1	29	30.4	30	29.8	29.4	29.1	29.1	29.2	29.5	29.8
2	30.4	31	31	29.2	29.4	29.7	29.4	29.5	29.4	29.5
3	32	32.5	32	32	31.9	31.5	31.2	31.1	31	31
4	28	28.6	26	27	28.2	27	27.3	27	26.5	26
5	27.4	27.5	27.3	26.6	26	26.1	26.3	26.2	26.4	26.8
6	29	29.4	29.4	28.3	28.2	28.6	27.3	27.5	26.1	25.7
7										
8	27.3	27.3	27.3	27.3	27.8	27.9	27.2	27.3	27.8	28
9	27.1	27.3	27.4	27.4	27.3	27.5	27.3	27.5	28	28.3
10	27.2	27.2	27.3	28	28.1	28.2	28.4	27.4	27.6	27.3
11	26.1	26.1	26.2	26.3	26.4	26.3	26.4	27.3	27	26.8
12	27.7	27.7	27.4	27.5	27.3	27.7	27.3	27.5	27.4	27.7
13	28.3	28.4	28.1	28	27.5	27.2	27	27.1	27	27
14										
15	26.6	26.9	26.4	26.2	26	26.1	26.2	26.5	26.4	26.5
average	28.2	28.5	28.1	28.0	28.0	27.9	27.7	27. 8	27.7	27.7
S.D	1.6	1.9	1.9	1.6	1.6	1.5	1.4	1.4	1.5	1.6

Example 2:S6K1 deficient mice have reduced body fat

Mice were dissected to determine the cause of the lower body weight exhibited by S6K1 deficient mice described in Example 1. S6K1 deficient mice were shown to have less intra-abdominal fat pads. Each fat mass and organ mass was weighed from tissue removed from 6 -month old male mice. Dissection of S6K1 mice revealed a severe reduction of epididymal white adipose tissue fat relative to wild type mice (0.8% +/- 0.1% compared with 3.4% +/- 0.1%; the values are averaged values +/- standard error mean; S.E.M). Percentage brown adipose tissue/body weight was also reduced in S6K1 deficient mice (0.5% +/- 0.05 % compared with 1+/- 0.1%), whereas organ size was essentially unaffected (see Table III below). Similar results were found also in female mice.

Table III

Tissue	**Wild-type** (tissue weight/body(weight)	**S6K1 -/-** (tissue weight/body weight)
Liver	4.2+/-0.3%	4.6+/- 0.2%

Thymus	0.14+/-0.014%	0.18+/-0.01%
Muscle	0.5+/-0.02%	0.5+/-0.01%
Kidney	1.5+/-0.05%	1.9+/-0.07%
Lung	0.8+/-0.1%	0.8+/-0.1%
Heart	0.8+/-0.1%	0.7+/-0.05%
Testis	0.6+/- 0.02%	0.75+/- 0.04%
Spleen	0.33+/-0.05%	0.28+/-0.02%
Brain	1.25+/-0.03%	1.63+/-0.06%

The data establish that S6K1 -/- mice have reduced body white fat and brown fat relative to wild type mice.

Example 3: S6K1 deficient adipocytes are smaller

To establish why S6K1 deficient mice exhibit less fat, adipose tissue sections were stained with hematoxylin and eosin and visualized by 20-fold magnification using a histology microscope. Both epididymal white adipose tissue (WAT) and brown adipose tissue from S6K1 deficient mice exhibited smaller cell size when compared to wild type.

Cell density and size was quantified in the adipose tissue sections of WAT - cell numbers were counted within an area of 120 x 120mm on six sections from three individual animals for each genotype using ImageProPlus software (Media Cybernetics). The data is presented below in Table IV:

Table IVa (wild type cell size distribution)

Cell size µm2	Cell number
0-99	
100-199	1
200-299	0
300-399	0
400-499	3
500-599	3
600-699	9

700-799	6
800-899	7
900-999	12
1000-1999	157
2000-2999	136
3000-3999	122
4000-4999	87
5000-5999	63
6000-6999	41
7000-7999	25
8000-8999	21
9000-9999	4
>10000	6

Table IVb (wild type cell size distribution)

cell size µm2	cell number
0-99	
100-199	1
200-299	0
300-399	0
400-499	3
500-599	3
600-699	9
700-799	6
800-899	7
900-999	12
1000-1199	30
1200-1399	30
1400-1599	42
1600-1799	28
1800-1999	27
2000-2999	136
3000-3999	122
4000-4999	87
5000-5999	63
6000-6999	41
7000-7999	25
8000-8999	21
9000-9999	4
>10000	6

Table IVc (S6K1 -/- cell size distribution)

cell size µm2	cell number S6K 1ko
0-99	9
100-199	14
200-299	15
300-399	20
400-499	30
500-599	24
600-699	41
700-799	43
800-899	42
900-999	48
1000-1999	181
2000-2999	33
3000-3999	1
4000-4999	
5000-5999	
6000-6999	
7000-7999	
8000-8999	
9000-9999	
>10000	

Table IVd

	wt	S6K1ko
Average	3556	1001
% of weight	1	0.28

In summary, results from electron microscopy, histology and cell density/size analysis establishes that the reduction in fat in S6K1 deficient animals is due to a reduction in fat cell size.

Example 4: Diet is not the cause of less fat in S6K1 deficient mice

To establish whether S6K1 mice exhibited dietary differences over wild type mice, which would explain the reduction in fat in S6K1 mice, food intake per mouse was measured every other day for 15 days using normal or high fat chow. Irrespective of whether placed on normal or high fat diet, S6K1 deficient mice eat the same total amount of food as wild type (about 4.6 +/- 0.1 g food/mouse/day),

but compared to body weight, they eat much more (about 0.18 compared to 0.15 g food/g body weight/day).

Furthermore, body weight was recorded weekly in wild type and S6K1 deficient mice fed high fat diets (high fat diet, Research diet, D12492, 60% kcal fat). When S6K1 mice are placed on a high fat diet, absolute body weight gain of S6K1 deficient mice was about 10.5 g over the period of high fat diet feeding (from week 7 to 27 of age) compared to about 14.4 g in wild type mice. Given the smaller body size of the knockout, relative weight gain was similar between genotypes (58.9% increase of body weight in S6K1 deficient mice compared to 58.0% increase of body weight in wild type after high fat diet feeding for 5 months. Even though the relative percentage of body weight gain in S6K1 deficient mice was similar to wild type, they fail to put on fat to the same extent as wild type mice. Over a three-month period, wild type mice gained 0.1 g/g compared with 0.02 g/g fat/body weight by S6K1 deficient mice. Thus, even though S6K1 mice eat more, they do not put on fat to the same degree as wild type mice.

Example 5: S6K1 deficient mice exhibit impaired adipogenesis

To test whether S6K1 deficient mice exhibit impaired adipogenesis, adipocyte differentiation was induced essentially as previously described (Hansen et al., 1999, J. Biol. Chem., 274, 2386-2393) using wild type or S6K1 deficient mouse embryonic fibroblasts (MEFs). The passage number of MEFs was within one passage. For differentiation, 2-day post confluent cells (day 0) were treated with growth medium containing 1 µM dexamethasone (Sigma), 0.5 mM methylisobutylxanthine (Aldrich), 5 µg/ml insulin (Boehringer Mannheim), and Ciglitazone (thiazolinedione, PPAR agonist: BIOMOL, GR-205, 0.5 µM) for 2 days. From day 2, the medium contained 5 µg/ml insulin and Ciglitazone and renewed every other day. Oil red O staining: Oil red O staining solution (0.5% Oil red O in isopropyl alcohol solution-distilled water (60:40) was filtered. Cells were washed with PBS and stained for 30 min and then washed with distilled water

two times. Cells deficient in S6K1 showed much less staining establishing that mouse embryonic fibroblasts deficient in S6K1 have impaired adipogenesis.

What is claimed is:

1. A method of identifying an agent effective in treating weight disorders, said method comprising the steps of:

   i) incubating S6 kinase with a compound;

   ii) detecting S6 kinase activity; and

   iii) determining a compound-induced modulation in the S6 kinase activity relative to when said compound is absent, wherein an alteration of the S6 kinase activity in the presence of the compound is indicative of an agent effective in treating weight disorders.

2. The method according to claim 1, wherein said modulation is inhibition of S6 kinase 1 activity and said weight disorder is obesity or an overweight condition.

3. The method according to claim 1, wherein said modulation is activation of S6 kinase 1 activity and said weight disorder is an underweight condition.

4. The method of claim 1, comprising determining S6 kinase activity using S6 as a substrate.

5. A method of screening for an agent effective in treating weight disorders, the method comprising (a) contacting transcriptionally active cellular components with a nucleic acid encoding an S6K gene operably linked to a promoter sequence or an S6K promoter sequence operably linked to a reporter gene in the presence of at least one compound; and (b) detecting an effect of said compound on S6 kinase expression or S6 kinase promoter activity, wherein detection of a decrease or an increase in S6 kinase expression or promoter activity is indicative of an agent effective in treating weight disorders.

6. The method of claim 5, wherein said transcriptionally active cellular components and said nucleic acid is present in a cell.

7. The method of any one of the preceding claims, wherein said S6 kinase is S6 kinase 1.

8. A method of identifying an agent effective in treating weight disorders, said method comprising: (a) providing a non-human animal comprising an S6 kinase gene; (b) administering an agent to the non-human animal; and (c) determining whether weight loss or gain is affected relative to when said agent is absent.

9. An agent identified by any one of the preceding claims.

10. A method for reducing adipocyte size, said method comprising contacting an adipocyte with an effective amount of an S6 kinase 1 inhibitor.

11. A method for treating a weight disorder, comprising administering to a subject a pharmaceutically effective amount of an S6 kinase modulator.

12. The method of claim 11, wherein said S6 modulator is an S6K1 inhibitor and said weight disorder is obesity or an overweight condition.

13. A specific modulator of S6 kinase for the manufacture of a medicament for the treatment or prophylactic treatment of a weight disorder.

14. The modulator of claim 13, wherein said modulator is a specific inhibitor of S6 kinase 1 for the treatment or prophylactic treatment of an overweight condition or obesity.

15. A compound that is a modulator of S6 kinase activity for use in treating weight disorders.

16. The compound of claim 15, wherein said compound is an inhibitor of S6 kinase 1 activity, for use in the treatment of obesity or an overweight condition.

17. A method of diagnosing a predisposition to a weight disorder, comprising:

obtaining a sample from an individual,

detecting the level of S6 kinase activity in said sample

correlating a change in S6 kinase activity when compared to a normal control value or range of values with a predisposition to a weight disorder.

18. The method of claim 17, wherein said S6 kinase activity is S6K1.

19. The method of claim 18, wherein said change is an increase in S6 kinase activity when compared to a normal control value or range of values and said weight disorder is an overweight condition or obesity.

20. A method of evaluating treatments for weight disorders, the method comprising administering a therapeutic agent to a non-human animal comprising an S6 kinase gene and determining the effect of the agent on weight gain or loss.

ABSTRACT OF THE DISCLOSURE

Screening Methods for Agents Effective in Obesity

This invention provides screening methods for agents effective in treating obesity through specific inhibition of S6 kinase 1 activity. Also provided are methods of treating obesity by administering an effective amount of an inhibitor specific for S6 kinase 1.